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Physiological and environmental controls on the nitrogen and oxygen isotope fractionation of nitrate during its assimilation by marine phytoplankton

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Submitted in fulfilment of the requirements for the Doctor of Philosophy
University of Tasmania July 2014

Under a conjoint arrangement with Princeton University

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Abstract

Physiological and environmental controls on the nitrogen and oxygen isotope fractionation of nitrate during its assimilation by marine phytoplankton

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Nitrogen (N) is an essential nutrient for phytoplankton growth and its availability often limits primary production in the surface ocean. Quantifying the inputs, losses, and internal cycling of N is thus a major goal in marine biogeochemical research. The N isotope effect ($^{15}\varepsilon_{org}$) for nitrate assimilation is a key parameter in using N isotope distributions ($^{15}N/^{14}N$) to study the marine N cycle. Unexplained variability in its magnitude is a major source of uncertainty to N isotopic studies in the modern and past ocean. The ratio of the oxygen (O) and N isotope effects for nitrate assimilation ($^{18}\varepsilon_{org}$:$^{15}\varepsilon_{org}$) is also an important parameter; the association of nitrate assimilation with an $^{18}\varepsilon_{org}$:$^{15}\varepsilon_{org}$ near 1 is the cornerstone of studies using coupled nitrate N and O isotope measurements to separate co-occurring N cycle processes that have counteracting effects on the N isotopes alone. The association of nitrate assimilation with an $^{18}\varepsilon_{org}$:$^{15}\varepsilon_{org}$ near 1 is based on empirical evidence without full understanding of the physiological mechanisms generating the ratio. A better understanding of the controls on both the magnitude and ratio of N and O isotope effects for nitrate assimilation would strengthen environmental application of N and O isotopes.

Towards this goal, the individual N and O isotope effects for each fractionating step in nitrate assimilation (nitrate reduction, uptake, and efflux) were measured. The reduction of nitrate to nitrite is catalyzed by the intracellular enzyme eukaryotic assimilatory nitrate reductase (eukNR). An N isotope effect of 26.6 ± 0.2‰ and nearly equivalent N and O

fractionation were measured in two distinct forms of eukNR (the NADPH form in cell-free extracts from the fungus *Aspergillus niger* and the NADH form in cell homogenates from the marine diatom *Thalassiosira weissflogii*), suggesting these values will apply to the eukNR family as a whole. These are the first reliable N and O isotope effect measurements for an enzyme that catalyzes the rate-limiting step in nitrate assimilation for all eukaryotic plants and algae.

The N and O isotope effects for nitrate uptake and efflux were measured in the marine diatom *T. weissflogii*. Nitrate uptake and efflux were isolated from nitrate reduction by growing the cells in the presence of tungsten, which substitutes for molybdenum in assimilatory nitrate reductase, yielding an inactive enzyme. The N isotope effects for nitrate uptake and efflux were 2.0 ± 0.3‰ and 1.2 ± 0.4‰, respectively. The O isotope effect was 2.8 ± 0.6‰ for both uptake and efflux, yielding ratios of O to N isotopic fractionation greater than 1 for both processes. In sum, these results confirmed the existing physiological model for isotopic fractionation during nitrate assimilation where the isotope effect associated with intracellular nitrate reductase is high, the isotope effect associated with nitrate uptake is low, and the magnitude of $^{15}\varepsilon_{org}$ depends on the degree to which intracellular fractionation by nitrate reductase is expressed outside the cell by efflux. They also provided the first step in establishing how the $^{18}\varepsilon_{org}$:$^{15}\varepsilon_{org}$ near 1 for nitrate assimilation originates at the cellular level.

Finally, the whole cell N and O isotope effects ($^{15}\varepsilon_{org}$ and $^{18}\varepsilon_{org}$) for nitrate assimilation were measured in steady state cultures of *T. weissflogii* to assess how environmental and physiological parameters affected the extent of nitrate efflux and thus the magnitude of $^{15}\varepsilon_{org}$ and $^{18}\varepsilon_{org}$. Steady state cultures ensured $^{15}\varepsilon_{org}$ was not affected by transients in culture conditions and that $^{15}\varepsilon_{org}$ could be related to intracellular nitrate concentration and N and O isotopic composition. As observed in previous studies, $^{15}\varepsilon_{org}$ and intracellular [NO_3^-] increased under light limitation (a 3-fold and 5-fold increase respectively,

relative to results in non-limited cultures). $^{15}\varepsilon_{org}$ and intracellular [NO_3^-] were invariant under phosphate limitation. The ratio $^{18}\varepsilon_{org}$:$^{15}\varepsilon_{org}$ was near 1 under all conditions. In conjunction with previous results from iron- and temperature-limited batch cultures, these results suggest the N and O isotope effects for nitrate assimilation are (i) invariant under most environmental conditions and that (ii) irradiance may be the major driver of variability in $^{15}\varepsilon_{org}$ and $^{18}\varepsilon_{org}$ in the ocean.

The measurements also yielded insight into the regulation of nitrate efflux and suggest a role for intracellular NO_3^- storage in the environment. The near-constant magnitude of $^{15}\varepsilon_{org}$ observed under phosphate-limited growth suggests that the ratio of nitrate efflux to uptake remains 12-15% across a 4-fold increase in growth rate. The constant, non-zero efflux rate this implies suggests a component of efflux is inevitable and correlated with growth rate. The high $^{15}\varepsilon_{org}$ measured under light limitation show that efflux rates increase to 60% of gross nitrate uptake rates, suggesting that an additional component of efflux is correlated with high intracellular [NO_3^-]. The high intracellular [NO_3^-] observed under light limitation may suggest an adaptation to rapidly changing irradiance in the turbulent mixed layers of the surface ocean: excess intracellular NO_3^- storage may maximize assimilation rates and/or provide an electron sink to dissipate excess energy.

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